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DOI: <https://doi.org/10.4414/smw.2012.13618>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-68798>

Journal Article

Published Version

Originally published at:

Nischang, Marc; Gers-Huber, Gustavo; Audigé, Annette; Akkina, Ramesh; Speck, Robert F (2012). Modeling HIV infection and therapies in humanized mice. Swiss Medical Weekly, 142:w13618.

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Modelling HIV infection and therapies in humanised mice

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Summary

The human immunodeficiency virus (HIV) type-1 is a human-specific virus. The lack of a widely available small-animal model has seriously hampered HIV research. In 2004, a new humanised mouse model was reported. It was based on the intrahepatic injection of human CD34⁺ cord blood cells into newborn, highly immunodeficient mice. These mice develop a lymphoid system of human origin and are highly susceptible to HIV infection and showed disseminated infection, persistent viraemia and characteristic helper CD4⁺ T-cell loss. Here, we will briefly review the various existing humanised mouse models and highlight their value to the study of HIV infection.

Key words: *HIV-1 infection; HIV pathogenesis; humanised mice; BLT mice; SCID; human CD34⁺ cells; gene therapy; CCR5; antiretroviral treatment; microbicides; HIV pre-exposure prophylaxis (PrEP)*

The HIV situation globally

The UNAIDS report on the Global AIDS Epidemic 2010 optimistically announced that the HIV pandemic had peaked in the preceding two years. Nevertheless, the numbers are shocking: an estimated 33.3 million humans – 0.8% of all adults 15–49 years old – are infected, and more than 1.8 million people died in 2009 (http://www.unaids.org/globalreport/Global_report.htm). More hopefully, the number of people newly infected with HIV declined by nearly one-fifth over the last decade (1999, 3.1 million; 2009, 2.6 million). This decline is based on more widely applied “safer sexual practices” and reductions in mother-child transmission. The UNAIDS vision is Zero New Infections, Zero Discrimination, Zero HIV-associated Deaths. The goal is to halt and reverse the spread of HIV.

A human-specific virus: a challenge for *in vivo* studies

HIV specifically infects human cells. Even cells from chimpanzee, a very close relative of humans, are only

somewhat permissive to HIV infection [1]. Human host factors are critical for the virus throughout its entire replication cycle (fig. 1). For example, to enter a cell and begin its replication cycle, HIV engages a receptor complex of CD4 and a chemokine receptor, either CCR5 or CXCR4 [2, 3]. However, expressing human CD4 on murine cells does not make them permissive to HIV. Other human-specific factors, such as the human chemokine receptors, are needed.

Over the last three decades, human transgenes essential for HIV replication were expressed together in rodent cells, but the cells were still not permissive [4]. Furthermore, human transgenes were expressed in rodents in an attempt to generate HIV small-animal models. These models confirmed the human-specific nature of HIV and the *in vitro* data. No replication was observed in mice expressing human CD4 [5], CD4 and CCR5 [6] and Cyclin CDK9 [7], and rats transgenic for human CD4 and CCR5 replicated HIV only at very low levels for limited times [8, 9].

Other *in vivo* studies attempted to create models based on creating chimeric HIV strains. This approach relies on engineering a distinct HIV gene in a species-specific retrovirus, which despite the HIV transgene, replicates vigorously in the original species (e.g., simian-immunodeficiency virus [SIV] engineered to express the HIV envelope [SHIV]) [10]. The use of SHIV in monkeys allowed key questions about immune responses to vaccine constructs expressing various HIV gene encoded proteins to be addressed [10]. However, use of monkeys as animal models is restricted to specific questions with a narrow focus and cannot recapitulate the overall complexity of HIV, since the biological properties between SHIV, SIV and HIV are quite distinct. Finally, HIV-encoded gene products were expressed entirely as transgenes in mice [11–15]. These studies provided insight into the pathogenic potential of HIV gene products. However, they were expressed universally at high levels, and it is difficult to assess the significance of the resulting data since the dynamic nature of true HIV replication is lacking.

The requirements for a mouse model to study HIV infection

Faithfully modelling any human disease in an animal is difficult. Does the model replicate enough key features of the disease to allow us to conduct experiments?

Many of the key features of HIV infection are known. The main route of HIV transmission is vaginal or rectal intercourse. In acute HIV infection, a massive productive infection causes cell death in the lymphatic system, most prominently in the gastrointestinal tract. About 3–5 weeks after acute HIV infection, the levels of HIV RNA decline and the specific anti-HIV CD8⁺ T-cell response begins. Unlike in acute infection, fewer than 1% of CD4⁺ T cells are productively infected in the chronic phase [16], a number that cannot fully explain the progressive immunodeficiency. Poorly understood bystander effects seem to contribute to the overall cell loss [17], and sustained immune activation triggers it [18]. Combined antiretroviral treatment (cART) has been very successful in suppressing HIV RNA levels to below the limit of detection in about 90% of treated patients [19] and has resulted in a marked reduction of morbidity and mortality [20]. However, cART does not cure HIV. A small portion of HIV remains silent in long-lived cells, such as the quiescent memory CD4⁺ T-cells [21]; these cells form a latent reservoir of HIV. Besides finding simpler and more efficient treatment strategies, major efforts are now aimed at eradicating the latently infected cells to eventual cure HIV, and to develop novel gene therapy approaches and vaccination strategies. Other efforts are focused on orally administered pre-exposure prophylactic measures using anti-retroviral drugs and finding effective topical microbicides that can prevent sexual transmission. Thus, the requirements for a HIV mouse model include the following:

- Permissiveness to replication-competent HIV with distinct co-receptor usage (i.e., CCR5- or CXCR4-tropic HIV strains), resulting in high-level viraemia, systemic viral dissemination and histopathology reminiscent of HIV disease in humans.
- Supporting long-term chronic infection, allowing monitoring of HIV infection over time.
- Susceptibility to natural transmission modes of HIV, including vaginal and rectal routes.

- Displaying gradual depletion of CD4⁺ T-cell numbers during HIV infection.
- Activation of the immune system to lead to HIV-specific immune responses.
- Establishment and maintenance of an HIV latent reservoir.
- Allow development and testing of anti-HIV therapeutic and prevention strategies.

Humanised mice in general

The generation of humanised mice involves either the expression of human transgenes or the transplantation of human tissue into immunodeficient mice. However, as mentioned above, even constitutive expression of multiple human transgenes has not rendered mice fully permissive to HIV infection.

The human-PBL-SCID and foetal thy/liv SCID mouse model

Transplantation of human tissue into immunodeficient mice without rejection was first reported in the early 1980s. This became possible with the identification of a spontaneous mutation of the *Prkdc* gene in mice, which results in the complete lack of T and B cells and consequently in severe combined immunodeficiency (C.B.-17 SCID/SCID [SCID]; descriptions of the various mouse strains, see box) [22]. The *Prkdc* gene encodes for the catalytic subunit of a DNA-dependent protein kinase that is needed for V(D)J recombination in developing T and B lymphocytes. The two early humanised (Hu) mouse models were the foetal thymus/liver (thy/liv) SCID-hu mouse [23, 24] and the hu-PBL-SCID (PBL, peripheral blood leucocytes) mouse [25]. The foetal thy/liv SCID-hu mouse model is based on surgical placement of foetal thymus/liver tissue under the renal capsule. At 4–6 months post-implantation, foetal thymus/liver tissue forms a conjoint organoid that resembles human thymus and sustains T-cell lymphopoiesis for over a year [23]. The system is susceptible to HIV infection, but in the absence of robust peripheral human leukocyte reconstitution, samplings to analyse the infected human cells are mainly restricted to the engrafted conjoint organoid. Also there is no multilineage human haematopoiesis in this model (table 1).

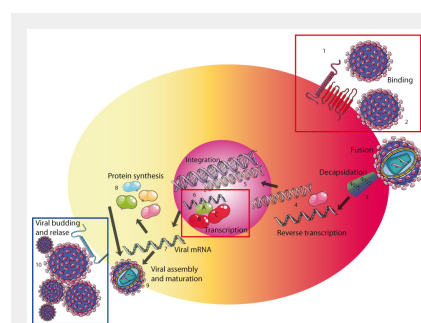


Figure 1

HIV-1 needs critical host factors for efficient replication. HIV binds to the HIV receptor complex of the human CD4 cell-surface molecule and a co-receptor, either CCR5 or CXCR4, via the HIV envelope glycoprotein 120 (HIV env gp120). After conformational changes in the HIV env gp41, viral host cell membrane fusion occurs (2). The next steps are the decapsulation (3) and release of the HIV RNA from the virus particle. Reverse transcription generates a viral complementary DNA (cDNA) based on the viral RNA template and using HIV's own reverse transcriptase (4). Once the cDNA is generated, the preintegration complex (PIC) is assembled, nuclear trafficking and integration of the viral cDNA into the host genomic DNA follow (5). Efficient transcription and elongation require formation of P-TEFb (positive transcription elongation factor b) consisting of Tat (6A), human cyclin-dependent kinase 9 (CDK-9) (6B) and cyclin T1 (6C), which binds to the nascent HIV transcripts. Fully or partially spliced HIV mRNA (7) is used to translate viral proteins. Unspliced HIV RNA is packaged into newly generated virions. Assembly of HIV proteins and RNA and budding takes place at the cellular membrane (9). HIV release is inhibited by murine tetherin at the cellular membrane because murine tetherin is insensitive to the viral protein Vpu, which inhibits human tetherin by directing its proteasomal degradation (blue frame). Human host factors critical for HIV replication are CD4, CCR5, CXCR4 and cyclin T1 (red frame). Additional human specific factors probably exist.

The hu-PBL-SCID mouse model is based on the intraperitoneal injection of human PBL [25] and is susceptible to HIV infection [26]. However, within days, human PBL injected into mice react against the murine disparity with a vigorous activation: their proliferation rate increases, and the CCR5 chemokine receptor and HLA-DR are upregulated [27–29], resulting in xeno-reactive T-cells [30]. Mice with significant blood T-lymphocyte chimerism suffer from high levels of graft-versus-host disease (GVHD) and mortality. Mice with no or transient T-cell chimerism have a low incidence [31]. Use of this model is limited mostly by the lack of *de novo* development of continuously differentiating human cells, activation status of the xenoreactive T cells and the GVHD (table 1).

New approaches for generating humanised mice

In 2004, a novel humanised mouse model was reported. It was based on transplanting human CD34+ haematopoietic progenitor cells (CD34+ cells) directly into the liver of newborn immunodeficient mice (Rag2^{-/-} γ_c^{-/-}) [32]. By 10 weeks after transplantation, the mice develop a lymphoid system of human origin with T cells, B cells, NK cells,

monocytes and dendritic cells. Notably, the T cells display a pattern of naive and memory cells and a Vβ repertoire similar to that of humans. The mouse mounts a specific antibody response against model antigens, such as pneumococcal and tetanus toxoid antigens, but the response is much weaker than that in humans (table 1).

This model is a significant step toward humanisation. Importantly, the mice lack the γ_c chain, which results in even more drastic immunodeficiency as compared with SCID mice. The γ_c chain is an essential component of the IL-2, -4, -7, -9, -15 and -21 receptors. Its absence severely compromises the development of immune cells, including NK-cell development, and thus their rejection potential against transplanted xenogeneic tissue. It also makes the mice less susceptible to lymphoma development. Indeed, the NOD-SCID IL-2Rγ-null mice are much more useful than the NOD-SCID mice for transplanting human tissue [33]. Notably, NOD-SCID IL-2Rγ-null mice show a similar degree of immunodeficiency as Rag1 or 2^{-/-} γ_c^{-/-} knock-out mice; they have been developed by crossing of SCID mice with non-obese diabetic (NOD) mice and mice deficient in the gamma c (γ_c) chain of the IL-2 receptor [34].

Table 1: Compilation of the various humanised mouse models.

Humanised mouse model	Engraftment	Cellular composition in reconstituted hu mice	Supports HIV infection with	Advantages	Disadvantages
hu-PBL SCID [25]	N/A	• T and B cells	• CCR5 and CXCR4-tropic strains [§] [26, 28, 62]	• Easy to generate (i.e. good access to PBLs) • Can immediately be used after transfer of PBLs	• No multilineage haematopoiesis • Limited time frame for experiments [62] • Strong activation of T-cells [27, 28] • Emergence of xeno-reactive T-cells (GVHD) [30]
Thy/Liv SCID hu [23]	N/A	• T cells • Single positive double positive and double negative Thymocytes	• CCR5 ^{-*} and CXCR4-tropic strains [24, 58, 59]	• Organoid of foetal thymus/liver tissue with sustained T-cell lymphopoiesis • Valuable to study certain pathogenic aspects (see text)	• Surgical skills needed • Human foetal tissue needed • No multilineage haematopoiesis • Sampling mainly restricted to the organoid since lack of solid peripheral reconstitution • Lack of CCR5 expression on intrathymic T progenitor cells
Rag2^{-/-} γ_c^{-/-} [32]	++	• T and B cells • Monocytes • Macrophages • NK cells • DCs	• CCR5- and CXCR4-tropic strains [68, 69, 71]	• Long-term multilineage haematopoiesis • Specific antibody response to recall antigens [32] • Suited to study HIV pathogenesis [118, 131, 132], HIV latency [90], gene therapy and novel anti-HIV treatment approaches [90]	• Delay between transplantation of human CD34+ cells and development of lymphoid system of ~15 weeks.
NOG [38] or NSG [33]	+++	• T and B cells • Monocytes • Macrophage • NK cells • DCs	• CCR5- and CXCR4-tropic strains [40, 70, 131, 132]	• Higher reconstitution levels as compared to Rag mice [39, 41] • Suited for studying HIV pathogenesis [119, 123], HIV treatment [95, 98] and latency and gene therapy approaches [102, 107]	• Sensitive to irradiation
NOD/SCID ¶ -hu BLT [42] And NOD/SCID γ_c^{-/-} (NSG) BLT [133]	+++	• T and B cells • Monocytes • Macrophages • NK cells • DCs	• CCR5- and CXCR4-tropic strains [43, 77, 80, 88, 108, 115]	• Generation of adaptive immune responses [115] • Suited for studying HIV pathogenesis [43, 115], anti-HIV treatment [77, 80, 108], HIV latency [88] as well as novel gene therapies	• Two step procedure for generating BLT mice • Surgical skills needed • Human foetal tissue needed

N/A = not applicable

+ = good engraftment

++ = high engraftment

+++ = very high engraftment

[§] Infection using CXCR4 tropic HIV-1 strains only successful shortly after transfer of human PBLs.

* Controversial data concerning the permissiveness of foetal thy/liv SCID mice to infection with CCR5 tropic HIV-1 strains.

¶ For simplicity reasons, we put together these two subtly different models; in fact, BLT mouse using NSG background show a superior engraftment as compared to NOD/SCID BLT mice.

Modifications for improving the engraftment of human haemato-lymphopoietic tissue have been investigated, including the use of human foetal liver derived CD34+ cells, cultivating the CD34+ cells with a cytokine cocktail before transplantation [35], pre-conditioning the mice with busulfan instead of irradiation [36, 37], the use of different mouse strains, such as NOD/shi-scid/ γ_c null (NOG) [38] or NOD/SCID/ γ_c -/- (NSG) mice [39], the transplantation of CD34+ cells intravenously or into the bone marrow or the transplantation of CD34+ cells at older age of the mice [39]. (NSG and NOG mice are nearly identical except for the modification of the γ_c chain receptor; in both strains, triggering through the γ_c chain receptor is disabled: in NSG mice the receptor is completely knocked down, and in NOG mice the intracytoplasmic tail is truncated). NOG mice are especially vulnerable to developing lymphomas after irradiation; however, they yield similar engraftment results even when not irradiated [40]. Very importantly, the lifespans of humanised mice, except of irradiated NOG mice, appear to be similar to those of wildtype mice; the mice eventually die due to infirmity. NSG mice transplanted at birth with haematopoietic progenitor cells either from human foetal liver or from human cord blood gave the better engraftment than the Balb/c-Rag1-/- and C.B-17-scid/bg mice [41]. Similar data have been reported by Brehm et al. [39].

The BLT mouse deserves special mention [42]. BLT is an acronym for bone marrow liver thymic. In this model, foetal liver/thymus is placed under the renal capsule in 6–8-week-old immunodeficient mice as with standard SCID-hu mice. However, after 3 weeks, the mice are sublethally irradiated, and autologous human CD34+ cells are transplanted into the mice. These cells home to the bone marrow and also migrate to the scaffold generated by the initial transplantation of the human foetal liver/thymus tissue. In the BLT mice, engraftment of human lymphoid tissue is highly efficient, even to the gastro-intestinal tract [43]. The innate and adaptive immune responses appear to be generally more complete in the BLT mice than in humanised mice generated by transplanting human CD34+ cells alone [42]: BLT mice generate a human MHC-restricted T-cell response to Epstein Barr virus (EBV) and activated V β 2-TCR+ T-cells when dendritic cells present the superantigen toxic shock syndrome toxin 1 (TSST-1). Notably, TSST-1 specifically activates and induces the TCR V β 2+ cells to proliferate. Generating an adaptive immune response is facilitated by educating the human T-cells in an autologous thymic microenvironment. This is not the case in the other humanised mouse models, which have xenogeneic mouse thymic environments. To overcome this limitation, immunodeficient mice were generated expressing the human HLA class I genes [44]. Here, mice transplanted with HLA-matched cord blood cells supported the *in vivo* differentiation of functionally mature human cytotoxic lymphocytes associated with a wide spectrum of functional human T-cell subsets. The mice mounted an EBV-specific immune response upon challenge as quantified by tetramer staining and enzyme-linked immunospot (ELISPOT) assay.

Thus, introducing human HLA-class I transgenes significantly improved the humanisation of the mice. Similarly, a

new report demonstrated expression of class II (HLA-DR4) in NOD- Rag1 -/- / γ_c -/- mice and consequent improvement in T- and B-cell development and function [45]. Additional human transgenes critical for haematopoiesis have been introduced into the mouse strain backgrounds, and this action should result in a lymphoid system that even more closely approximates the human lymphoid system.

Humanised mice have also been used to study (1.) haematopoietic development, (2.) a variety of microorganisms, including EBV [42, 46], herpes simplex virus [47], Dengue fever [48, 49], influenza [50] and *Salmonella typhi* [51, 52], (3.) sepsis [53] and iv) virus-induced tumours [54, 55].

Irrespective of the strain, immunodeficient mice are prone to opportunistic infections and must be kept in optimized hygienic animal care facilities. Whether the humanisation protects mice from infections is not known.

Humanised mice for studying HIV infections

The hu-PBL SCID and foetal liv/thy SCID hu mouse models have been valuable for the study of HIV infection, including immune responses (e.g., the effect of vaccination with vaccinia gp160 and recombinant gp160 [56]), *in vivo* drug testing [57–59], anti-HIV effects of CD8+ cytotoxic T-cells [60] and neutralising antibodies [61], virulence of HIV isolates [62], and the significance of distinct HIV accessory proteins on virulence [63, 64], and viral latency [65]. However, these models have several limitations. Most importantly, they lack multilineage haematopoiesis and the capacity to generate an effective human immune response (table 1).

The “new generation” of humanised mice has a number of positive aspects, such as multilineage haematopoiesis, no or very rarely graft-vs-host disease, a longer lifespan of the mice, and the generation of some immune responses (table 1). In the next sections, we will focus exclusively on these new humanised mouse models. Reviews comparing the properties of the various humanised mouse models based on the use of either Rag, NSG or NOG mouse strains have recently been published [66, 67]. In this review, we focused primarily on the overall value of humanised mice for studying HIV infection and specified only the mouse strain used when clear differences were described as related to HIV infection or pathogenesis.

Humanised mice support high-level viraemia

The new humanised mice support high levels of HIV infection with either CCR5- or CXCR4-tropic strains [36, 68–71]. Plasma HIV RNA copy numbers of 10^4 – 10^5 /ml in those mice are similar to the levels found in HIV-infected humans (note, that HIV replication can be easily monitored by repetitive sampling of peripheral blood). HIV-infected cells were detected in the spleen, lymph nodes, thymus and lungs, indicating dissemination of the virus. Unlike hu-PBL SCID mice, humanised mice sustain high-level viral replication for more than a year [35]. Depending on the virulence of the HIV strain used, the mice show distinct CD4+ T-cell depletion rates over time. Initial reports noted either very limited or no HIV-specific humoral immune responses [68, 70]. Importantly, expression of the HIV co-receptors

CXCR4 and CCR5 on engrafted and differentiated human immune cells was similar to that seen in humans [68, 70, 71]. Co-receptor expression in human CD4+ T cells is the major determinant of HIV tropism *in vivo* [72]. Indeed, as seen in HIV-infected human, disseminated infection in humanised mice with CCR5-tropic strains leads preferentially to infection and depletion of CD4+ memory T lymphocytes [73]. CCR5 is expressed mainly on memory T lymphocytes and is absent from naive T cells.

Humanised mice for studying sexual transmission and its prevention

A prerequisite for studying HIV sexual transmission in humanised mice is the engraftment of the female reproductive tract and/or the gastro-intestinal tract with virus susceptible human cells. Both humanised Rag1-/- γ_c -/- mice and Rag2-/- γ_c -/- mice, as well as BLT mice, are well engrafted with human cells in the vagina [74–77], and vaginal HIV transmission is efficient in all these three new mouse models. Like the human gut, the mouse small intestines include abundant Peyer's patches and the large intestines are populated with lymphoid follicular aggregates with human T and B lymphocytes, macrophages and DC [43, 74]. Here, memory T cells with prominent expression of CCR5 are permissive to CCR5-tropic strains. BLT mice also show human CD4CD8 $\alpha\alpha$ cells, a T-cell subset present only in the gut-associated lymphoid tissue [77]. These mice respond with disseminated HIV infection subsequent to either rectal or vaginal infection with cell-free HIV [43, 74, 75, 77].

However, efficient engraftment of the gastrointestinal tract of Rag2-/- γ_c -/- mice with human cells appears to depend on the protocol used: mice transplanted with CD34+ cells derived from human foetal liver and cultured overnight with IL-3, IL-6 and stem cell factor showed human cell engraftment in the gut [74]. This is not the case in mice transplanted with uncultured CD34+ cells derived from cord

blood [78]. The latter also differed in their susceptibility to rectal HIV challenge [74, 78].

Humanised mice represent a very significant advancement for evaluating novel microbicides for preventing HIV infection and very nicely complement the much more expensive monkey models. Indeed, several recent studies demonstrated the utility of these models for testing oral and topical pre-exposure prophylaxis strategies with different anti-HIV drugs (e.g., Tenofovir, Maraviroc, Raltegravir) currently on the market [77, 79–82] or with compounds in development [83]. In particular, topical application of the CCR5 antagonist Maraviroc formulated as a gel prevented HIV vaginal transmission [81], and the novel CD4 aptamer-siRNA chimeras [83] showed partial protection.

Studies of anti-retroviral treatment strategies

A few reports have noted the utility of the new humanised mice for evaluating antiretroviral therapies [84–89]. We made a major effort for defining a gold standard for ART in humanised mice by first examining the pharmacokinetic of a number of anti-retroviral compounds [90]. In this work we showed efficacious anti-retroviral treatment when the anti-retroviral compounds were added to food pellets or when long-acting drugs were used [90]. We also demonstrated emergence of resistance in insufficiently treated mice, and viral rebound from previously undetectable levels after cART interruption, confirming a latent reservoir as reported recently [85, 86, 88, 91]. Thus, humanised mice represent a highly valuable model for pre-clinical proof-of-concept studies to evaluate novel anti-retroviral compounds and to study latency that closely approximates the status of HIV-infected humans treated with cART.

Several studies also evaluated novel molecules for suppressing HIV *in vivo* in these new mouse models [89, 92–98]. They involved studies investigating the potential of Tat peptide analogues for inhibiting HIV replication (89) as

Box		
Human host factors	Definition	Function
CD4	Cluster of differentiation 4	HIV binding-entry receptor, expressed in T-helper cells, macrophages/monocytes and dendritic cells
CCR5	C-C motif-chemokine receptor 5	Transmembrane chemokine receptor, important for cytoskeletal reorganisation and cell motility, HIV co-receptor
CXCR4	CXC motif-chemokine receptor 4	Transmembrane chemokine receptor, chemotactic activity for lymphocytes, HIV co-receptor
Cyclin CDK9	Cyclin dependent kinase 9	Cell-cycle regulator, binds Tat and cyclin T1, required for RNA polymerase II transcriptional elongation of HIV
Mouse strains	Definition	Description
C.B.-17 SCID/SCID	Severe combined immunodeficiency (SCID)	Mutation in the <i>Prkd</i> gene (lack of B and T cells)
human-PBL-SCID	PBL: peripheral blood leucocytes	Intraperitoneal injection of human PBL into SCID mouse
Rag1 ^{-/-} γ_c ^{-/-} Rag2 ^{-/-} γ_c ^{-/-}	Deficiency in the recombinase activating gene 1 or 2 and the common γ chain of the IL-2 receptor	Lack of B, T and NK cells
NOD	Non-obese diabetic mouse	Reduction of NK cell activity
NOD/SCID	NOD and SCID mouse	Lack of B and T cells and NK cell activity
NSG (NOD/SCID/ γ_c ^{-/-})	NOD/SCID mice with entire knock-out of the common γ_c chain receptor	Lack of B, T and NK cells and block of the maturation and activity of these cells
NOG (NOD/SCID/ γ_c ^{-/-})	NOD/SCID mice with knockout of the intracytoplasmic tail of the common γ_c receptor	Lack of B, T and NK cells and block of the maturation and activity of these cells
BLT	Bone marrow, liver, thymus	NOD/SCID or NOD/SCID/ γ_c ^{-/-} mice transplanted with foetal liver, thymus and CD34+ cells

well as the effects of silencing (si)RNAs directed against viral proteins (e.g., Tat, Rev, Vif) or CCR5 that were delivered either by aptamers binding to the HIV envelope glycoprotein [92], dendrimer nanoparticles [93], single-chain antibodies binding to CD7 [95] or by immunoliposomes targeting the lymphocyte function-associated antigen-1 (LFA-1) [94]. The gp-120-binding aptamers targeted productively infected T cells specifically, and the single-chain antibodies and immunoliposomes targeted the white blood cells independently of HIV infection. In all of these *in vivo* studies, HIV replication was significantly suppressed. The humanised mice are also useful for studying the protective effect of distinct broadly neutralising antibodies delivered either by antibody-expressing cells administered as “backpacks” [96] or by adeno-associated virus-based vectors [97].

Studying gene therapeutic approaches for HIV/AIDS

Humanised mice represent a unique option to explore haematopoietic stem cell-based gene therapy strategies. Gene manipulation of human CD34+ cells to modulate host factors is also very attractive for HIV pathogenesis studies. Immunodeficient mice have long been used to assess the ability of gene-transduced human CD34+ cells to differentiate into various cellular subsets. In 1994, retroviral vector-transduced human CD34 cells were shown to differentiate into mature T-cell subsets in SCID-hu grafts [99]. Later, long-term engraftment of human CD34+ cells transduced with an HIV vector was demonstrated in NOD/SCID mice; 4–10% of the human cells were transduced [100]. NOD/SCID and the NSG mice seem to engraft similar numbers of transduced human CD34+ cells [101]. However, when using bone marrow from those mice for secondary reconstitutions in mice on the same background, human tissue from NSG mice engrafted far better than that from NOD/SCID mice, pointing to increased numbers of long-term SCID repopulating cells. These features are favourable for studying long-term transgene expression and the analysis of retroviral-insertion sites in primary and secondary transplanted NSG mice.

These findings, along with the higher levels of human tissue engraftment, suggest that the “novel generation” of humanised mice will be very useful for studying gene therapy approaches and examining distinct genes for their pathogenic effects in various settings. In addition, zinc-finger nuclease-mediated gene engineering is another very promising gene-engineering technology that has been explored in these mouse models [102].

A vast body of literature describes various approaches to control HIV by *ex vivo* gene therapy [103, 104]. Gene therapy approaches for HIV/AIDS i) target host factors critical for HIV replication or ii) HIV genes mandatory for HIV replication (e.g., Nef) [105], iii) introduce novel gene constructs (e.g., Trim5-cyclophilin fusion protein that inhibits HIV replication) [106], or iv) employ a broadly neutralising anti-HIV antibody [107]. The value of humanised mice for studying genetically altered human CD34+ cells to treat HIV is nicely illustrated by several studies, such as those targeting CCR5 with siRNA- or shRNA-mediated silencing [108, 109] or by zinc finger-mediated excision [102]. Notably, the HIV receptor complex consists of CD4

and either the HIV co-receptor, CCR5 or CXCR4 [72]. CCR5-tropic strains are transmitted and predominate until late-stage disease [110]. CXCR4-tropic strains emerge in advanced stages of HIV disease in about 50% of HIV-infected patients and seem to accelerate the immune deficiency [111]. Silencing CCR5 has been mentioned as a potential “cure” for HIV. A recent report described an HIV-infected patient who suffered from acute myeloid leukaemia and who was transplanted with human CD34+ stem cells lacking CCR5. Strikingly, the patient appears to be fully cured since HIV did not rebound subsequent to interruption of cART [112]. While it involved only a single patient, this report gives credence to genetic approaches targeting CCR5.

Introducing a lentiviral construct silencing CCR5 into human CD34+ cells resulted in a clear reduction of CCR5 on the target cells of HIV *in vivo* [108]. The engineered progenitor cells showed long-term haematopoietic repopulating capacity by secondary transplantation. Notably, the genetically engineered progeny cells behaved identically as the controls. *In vitro* the engineered cells were resistant to HIV infection with CCR5-tropic strains. Zinc finger-mediated excision of CCR5 in CD34+ cells resulted in progeny cells lacking CCR5, and the mice showed lower HIV replication and prevention of CD4+ T-cell loss *in vivo*, as compared to control mice [102]. Very importantly, HIV infection resulted in a selection of cells resistant to HIV over time. This technology demonstrated cells with long-term haematopoietic repopulation capacity. Other genetic approaches have focused on T cells and engineered HIV-resistant CD4+ T cells with CXCR4-specific zinc finger nucleases [113].

These studies clearly indicate that humanised mice are a very promising tool for exploring gene engineering approaches to treat and/or cure HIV. The major hurdles will not be the identification of targets rendering cells resistant to HIV but to achieve sufficiently high numbers of genetically engineered CD34+ cells, the migration of transduced cells to the niches of haematopoietic stem cells, preventing the insertional risk favouring neoplastic transformation or off-target effects (e.g., activation of the innate immune response). Here, too, the humanised mouse model will be a versatile tool for exploring these questions.

Humanised mice for generating an HIV-specific immune response

Inducing a robust HIV-specific immune response was reported in NSG mice reconstituted with human CD34+ cells from newborns [114] and in the BLT mouse model [115]. Both papers reported HIV-specific CD4+ and CD8+ T-cell responses with overlapping HIV peptide pools [114, 115] or ELISPOT assays [115]. The relevance of the CD8+ T cells in constraining HIV replication in humanised mice was illustrated by a significantly higher replication rate when CD8+ T cells were depleted [114]. The humanised mice also developed humoral immune responses against HIV [115]. However, antigen-specific immune responses in humanised mice seem to take longer to develop than in adult humans infected with HIV, possibly reflecting the lack of full maturity of the human immune systems of these mice after reconstitution [115]. In previous work, such sol-

id HIV-specific antibody immune responses were not reported. The analysis might have been performed too soon after HIV infection. In view of limited number of studies, we still do not know if the current generation of humanised mice is suited for studying antigen-specific immune responses and, in particular, vaccine approaches. In mice reconstituted directly with human CD34⁺ cells the selection of T cells is done by murine thymic stromal cells. The subsequent generation of an antigen-specific immune response, however, is based on the processing of antigens by human antigen-presenting cells in the humanised mouse model and therefore might be suboptimal.

HIV evolution over time

HIV's diversity is one of its main features. It is also a key element for immune escape and emergence of resistance to ART. HIV's diversity is due to the inaccuracy of the HIV reverse transcriptase, hyperamination of the nascent DNA strand during reverse transcription by the members of the APOBEC family, and recombination events between distinct HIV strains. Indeed, the genotypic and phenotypic changes in the viral envelope gene in humanised mice infected with a distinct HIV strain, JR-CSF, showed the mean rate of divergence of viral populations over 44 weeks similar to that in humans [116]. They noted a disproportionate number of guanosine-to-adenosine transitions in the HIV envelope, indicating that APOBEC3G is active in this model. Furthermore, a number of substitutions in the envelope gene were identified.

HIV immune activation and dysfunction

Sustained immune activation is the major trigger of HIV-associated immunodeficiency. Various mechanisms, such as disruption of the gastrointestinal tract barrier during acute HIV infection [117] or various HIV accessory gene products, may contribute to the HIV-associated immune activation [18]. Immune activation is also observed on the CD4⁺ and CD8⁺ T cells in HIV-infected humanised mice [115, 118]. We used the humanised mice to study the role of macrophages in immune activation [118]. We found that HIV infection results in a disturbed phagocytosis by macrophages. Notably, macrophages are essential for clearing bacterial products. We concluded that disruptions of the gastrointestinal tract barrier, together with the macrophage dysfunction, are a main element of higher blood levels of bacterial products and thus in HIV-associated immune activation.

Immune activation affects also the PDL1-PD1 axis (PDL = programmed death ligand). The inhibitory receptor PD-1, which indicates exhaustion of T cells, was increased on the T cells in HIV-infected humanised mice, reminiscent of the findings in humans [115]. Ongoing studies are examining the benefits of blocking the PD-1 pathway.

The presence of various immune cells, such as plasmacytoid dendritic cells (pDC) and T-regulatory cells, in humanised mice presents a unique opportunity to assess their effects on HIV infection and vice-versa (i.e., HIV's effect on them). For example, rapid infection and activation of pDCs were seen in HIV-challenged humanised mice [119]. Their activation correlated with activation of CD4⁺ T cells and their apoptosis. While CD4⁺ T cells were depleted,

pDCs were maintained but functionally impaired. The presence of T-regulatory cells in these mice may help to dissect their role in HIV infection. These cells are preferentially targeted by HIV during acute HIV infection in these mice [120].

CNS invasion by HIV

AIDS-related dementia occurs in about 30% of HIV-infected patients with advanced immunodeficiency [121]. AIDS dementia is characterised by the immigration of macrophages, formation of microglial nodules, and generation of multi-nucleated giant cells, most likely due to viral induced fusion between microglial cells and/or macrophages. HIV-infected humanised mice show pathologic anomalies in the brain reminiscent of those in HIV-infected patients with AIDS dementia. In particular, activated human blood-borne macrophages migrate into the brain. Human cells enter into the brains more quickly in HIV-infected mice than in control mice. Productively infected macrophages and cells of lymphocyte morphology are found in the meninges and perivascular spaces [122]. Strikingly, CD8⁺ T-cell depletion aggravated the pathological findings, suggesting that CD8⁺ T cells could subdue HIV infection to some extent. Using advanced neuroimaging and post-mortem examination, HIV-infected mice show a loss of neuronal integrity [123]. These data are encouraging: humanised mice represent a valuable tool for examining mechanistic and therapeutic aspects of HIV-associated dementia. However, as reiterated by the study authors, additional studies are needed for a more detailed characterisation and validation of the neuronal damage associated with HIV infection in this mouse model [124].

Future generations of humanised mice

Despite the advances made in humanised murine models, the reconstituted lymphoid system still lacks a well-elaborated lymphoid architecture. This is partially explained by the lack of human cytokines critical for haematopoiesis and/or by insufficient interactions between cells of the murine stroma and human haematopoietic cells. The less than optimal lymphoid architecture and the education/selection of T-cells on a murine thymic scaffold result in a rather modest adaptive immune response. As outlined above, transplantation of HLA-matched cord blood into a mouse strain transgenic for human HLA gives more robust specific T-cell response. To improve the humanisation of mice, additional human transgenes critical for haematopoiesis are introduced into mice.

The knock-in of human thrombopoietin (TPO) into Rag2^{-/-}γ_c^{-/-}, which is essential for the expansion and maintenance of HSC [125], resulted in a higher level of engraftment and an increase in the breadth of multilineage haematopoiesis with higher number of myeloid cells than in control mice expressing the murine TPO [126].

Other knock-in (KI) genes examined for improving the humanisation were human IL-3 in concert with human granulocyte macrophage cytokine stimulating factor (GM-CSF) [127] and colony stimulating factor-1 (CSF-1) [128]. In IL-3/GM-CSF KI mice, transplantation of human progenitor cells resulted in a more pronounced inflammatory

reaction in response to intraperitoneal administration of lipopolysaccharide than in controls. In addition, IL-3/GM-CSF KI mice had improved human myeloid immune reconstitution in the lung as exemplified by the presence of alveolar macrophages; the human alveolar macrophages mounted an innate immune response when challenged with influenza virus but could not control it. This model might be especially good for studying pulmonary diseases [127]. The knock-in of CFS-1 enhanced the differentiation of myeloid cells into monocytes and macrophages [128]. As outlined above, differences in the extent the various mouse backgrounds support human cell engraftment exist. Positional cloning identified alleles of the inhibitory receptor signal regulatory protein alpha (SIRPα) as a reason for the difference of engraftment levels between mouse strains [129]. In NOD mice, which have higher engraftment levels than other mice, SIRPα on murine macrophages showed enhanced binding to the human CD47 ligand. This enhanced binding inhibits phagocytosis of the xenograft and secretion of TNF-α by macrophages. Transgenic expression of SIRPα in Rag2-/-γc-/- increased the engraftment level of HSC to a level similar to NSG mice and improved the functionality of the immune system [130]. These next-generation mice will most likely become important assets for the research community. Furthermore, the step-wise progress made in humanisation methods will continue and will create an array of humanised mouse models appropriately suited to address specific research questions.

Funding / potential competing interests: Roberto F. Speck is supported by the Swiss National Science Foundation. No conflict of interest relevant to this article was reported.

Authors' contribution: Authors Marc Nischang and Gustavo Gers-Huber contributed equally.

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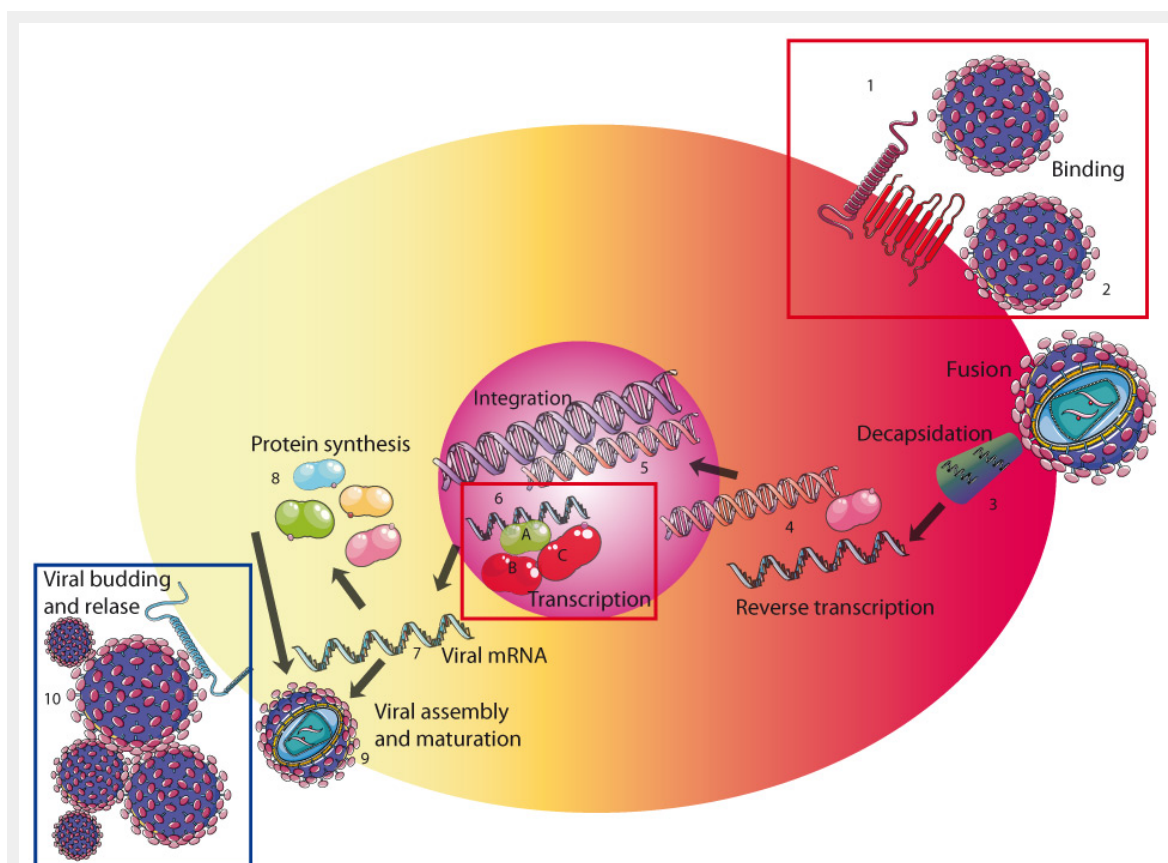
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Figures (large format)

**Figure 1**

HIV-1 needs critical host factors for efficient replication. HIV binds to the HIV receptor complex of the human CD4 cell-surface molecule and a co-receptor, either CCR5 or CXCR4, via the HIV envelope glycoprotein 120 (HIV env gp120). After conformational changes in the HIV env gp41, viral host cell membrane fusion occurs (2). The next steps are the decapsidation (3) and release of the HIV RNA from the virus particle. Reverse transcription generates a viral complementary DNA (cDNA) based on the viral RNA template and using HIV's own reverse transcriptase (4). Once the cDNA is generated, the preintegration complex (PIC) is assembled, nuclear trafficking and integration of the viral cDNA into the host genomic DNA follow (5). Efficient transcription and elongation require formation of P-TEFb (positive transcription elongation factor b) consisting of Tat (6A), human cyclin-dependent kinase 9 (CDK-9) (6B) and cyclin T1 (6C), which binds to the nascent HIV transcripts. Fully or partially spliced HIV mRNA (7) is used to translate viral proteins. Unspliced HIV RNA is packaged into newly generated virions. Assembly of HIV proteins and RNA and budding take place at the cellular membrane (9). HIV release is inhibited by murine tetherin at the cellular membrane because murine tetherin is insensitive to the viral protein Vpu, which inhibits human tetherin by directing its proteasomal degradation (blue frame). Human host factors critical for HIV replication are CD4, CCR5, CXCR4 and cyclin T1 (red frame). Additional human specific factors probably exist.